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1 Microwave-assisted extraction and hydrolysis: An alternative tool to pyrolysis for the analysis  
2 of recalcitrant organic matter? Application to a forest soil (Landes de Gascogne, France)

3  
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6  
7  
8 **Abstract**

9  
10 A comparison was made between the composition of the recalcitrant organic matter (ROM)  
11 isolated from a sandy forest soil as revealed by microwave assisted extractions and/or  
12 hydrolyses and by usual pyrolysis techniques. Successive microwave irradiation treatments  
13 were performed in H<sub>2</sub>O, 0.1 and 1 M HCl and 0.1 and 1 M KOH. At each step of the  
14 treatment the insoluble residue was examined via Curie point pyrolysis (CuPy) and Curie  
15 point thermally assisted hydrolysis and methylation (CuTHM). Sequential irradiation  
16 treatments resulted in ca 35% degradation of the ROM. Compounds released on microwave  
17 irradiation in H<sub>2</sub>O and in HCl were dominated by glucose, suggesting the occurrence of  
18 carbohydrate-containing molecular associations in the soil organic matter (SOM) which were  
19 not disrupted during acid hydrolyses and extractions as applied for the isolation of the ROM.  
20 The product distribution from the microwave irradiation in KOH showed an important  
21 contribution to the ROM from the higher plant polyesters cutin and suberin, and to a lesser  
22 extent from lignin. Different lignin-derived compounds were specifically released upon  
23 microwave acid or base hydrolyses. This suggested that two types of lignin monomers, ether  
24 or ester linked, occurred in the ROM. The changes observed in the composition of the CuPy  
25 pyrolysates of the residues from the different microwave hydrolyses are consistent with the  
26 near complete removal of carbohydrates by microwave HCl hydrolysis. The changes observed  
27 in the composition of the CuTHM pyrolysates of the residues from the different microwave  
28 acid and base hydrolyses are in agreement with a major release of cutin- and suberin-derived  
29 compounds upon microwave KOH hydrolysis. The CuPy and CuTHM pyrolysates of the final  
30 residue consists predominantly in lignin-derived compounds. This study emphasizes the  
31 potential of microwave-assisted hydrolyses to give a better estimate of the actual contribution

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32 of cutin to the ROM than pyrolysis. However, this technique appears to be unable to  
33 completely release the lignin-based constituent of the ROM. Microwave irradiation appears to  
34 provide great potential as a tool for extraction and chemical characterization of complex OM  
35 and could be an attractive additional technique to pyrolyses.

36

## 37 **1. Introduction**

38

39 Soil organic matter (SOM) is one of the major carbon pools playing an important role in the  
40 global carbon cycle. It contains a chemically recalcitrant (i.e. inert towards drastic laboratory  
41 acid hydrolyses) organic matter fraction (ROM). This recalcitrant, non-hydrolysable SOM  
42 fraction may account for a substantial part of the total organic carbon of the soil (Poirier et al.,  
43 2002, 2003; Quénéa et al., 2005a, 2006a; Mikutta et al., 2006) and can contribute to the stable  
44 carbon pool in the soil (Paul et al., 1997, 2006; Mikutta et al., 2006). The inherent or acquired  
45 recalcitrance of the ROM fraction and consequent long mean residence time in the soil might  
46 contribute to the potential role of SOM as a sink for atmospheric CO<sub>2</sub>. The chemical  
47 composition and origin of ROM have been chiefly studied using spectroscopic and pyrolytic  
48 methods (Poirier et al., 2002, 2003; Naafs, 2004; Quénéa et al., 2005a, 2006a, b). Few  
49 chemical degradation studies have been applied to characterise its composition (Quénéa et al.,  
50 2005b; Naafs, 2004; Winkler et al., 2005). These studies have pointed out the diversity of  
51 aliphatic structures making up the ROM network and the important role of ester functions to  
52 link these constituents. Aliphatic constituents are usually considered to derive predominantly  
53 from the biopolyesters cutin and/or suberin (Quénéa et al., 2005a,b) or from cutan and/or  
54 suberan (Augris et al., 1998; Naafs, 2004), the recalcitrant biopolymers originating from plant  
55 cuticles and suberized plant cell walls respectively (Nip et al., 1986; Tegelaar et al., 1995).  
56 Some of the pyrolytic studies have also pointed out the significant preservation of  
57 polysaccharide-type materials in the ROM (Quénéa et al., 2005a, 2006b). However, one of the  
58 main drawbacks of the usually on-line pyrolytic techniques performed is the lack of  
59 quantitative data about abundances of compounds released upon pyrolysis.

60 Microwave assisted extraction of organic compounds from matrices such as soils, seeds or  
61 food was introduced by Ganzler et al. (1986). This extraction technique has been extended to  
62 environmental analysis of contaminants in soils, sediments or water and to the extraction of  
63 natural products (e.g. Letellier and Budzinski, 1999; Camel, 2000). Microwaves are high  
64 frequency electromagnetic waves which are strongly absorbed by polar molecules.  
65 Absorption results in rapid and intensive dielectric heating. Microwave systems using closed

66 vessels can operate at elevated temperature and pressure and temperature of solvents  
67 submitted to microwave irradiation can be raised above their boiling point (e.g. Letellier and  
68 Budzinski, 1999). The complex macromolecular recalcitrant fraction of SOM contains polar  
69 constituents and strong localised heating can be expected to occur at these polar targets under  
70 microwave irradiation. This could result in extraction and/or release of some of the  
71 constituents of the matrix, opening up a new analytical possibility for obtaining information  
72 on the chemical composition of such macromolecular material.

73 In this paper a comparison was made between microwave assisted extraction and/or  
74 hydrolysis and pyrolytic methods for the chemical characterisation of recalcitrant organic  
75 matter. Capabilities of the two methods were illustrated by analysis of the ROM isolated from  
76 a sandy forest soil. The compositions of products released after neutral, acid and base  
77 microwave irradiation treatments were analysed. We compared these compositions to those of  
78 products released upon standard Curie point pyrolysis (CuPy/GC-MS) and Curie point  
79 thermally assisted hydrolysis and methylation (CuTHM) with tetramethylammonium  
80 hydroxyde (TMAH). The comparison was used to evaluate the potential of the two methods  
81 for chemical characterization of complex organic matter.

## 82 **2. Materials and methods**

83

84 All chemicals used were analytical grade.

85

### 86 *2.1. Sample*

87

88 The ROM sample was isolated from a soil collected from a maritime pine (*Pinus pinaster*)  
89 forest. The dominant forest undergrowth was composed of ferns (*Pteridium aquilinum*) and  
90 perennial grasses (*Molinia coerulea*; Jolivet, 2000). The isolation protocol and bulk features  
91 of the ROM have been previously described (Quénéa et al., 2005a). Briefly, lipid- and humic  
92 substance-free soil was submitted to stepwise acid hydrolyses using trifluoroacetic acid and  
93 hydrochloric acid. The hydrolysed material was demineralised using HCl/HF treatment. The  
94 ROM was recovered as the insoluble residue remaining after neutralisation and extraction  
95 with CHCl<sub>3</sub>/MeOH (2/1, v/v). The ROM accounted for 1.6% of the whole soil, i.e. about 34%  
96 of the total initial carbon. (Quénéa et al., 2005a). Its elemental composition was 58.7% C;  
97 4.2% H; 1.0% N; 4.5% ash (Quénéa et al., 2005a).

98

### 99 *2.2. Microwave assisted extraction and hydrolysis*

100

101 An outline of the sequential microwave assisted extractions and hydrolyses is shown in Fig. 1.  
102 The treatments were performed with a single-mode CEM Discover<sup>®</sup> microwave reactor at a  
103 frequency of 2450 MHz (0-300 W) in closed reaction vessels. The temperature was measured  
104 with an infrared sensor outside the reaction vessel. The samples were subjected to 20  
105 irradiation cycles. An irradiation cycle consisted in an irradiation period of 40 s followed by a  
106 phase of cooling, without transfer of microwave energy, for 1 min.

107 The ROM (ca. 100 mg) in 2 ml H<sub>2</sub>O was subjected to the irradiation cycles using 100 W  
108 microwave energy. The maximum temperature and pressure were 140 °C and 2.5 10<sup>5</sup> Pa  
109 respectively. After the irradiation cycles, the reaction vessel was cooled to room temperature  
110 with compressed air and the reaction mixture extracted at room temperature during 4 h with  
111 CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1 v/v; 30 ml). The reaction mixture was centrifuged at 1,400 g for 15 min.  
112 The extract **1** was dried under reduced pressure, treated with 4 M HCl in MeOH [prepared by  
113 mixing CH<sub>3</sub>COCl with MeOH (1:2.5 v/v)] for 1 h at 60 °C to esterify carboxyl groups and  
114 then with a mixture of pyridine/*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 10:1 v/v)  
115 for 10 min at 60 °C to convert hydroxyl groups to trimethylsilyl ethers.

116 The insoluble residue **1** from the CH<sub>2</sub>Cl<sub>2</sub>/MeOH extraction was dried, transferred to a reaction  
117 vessel and 2 ml 0.1 M HCl were added. The reaction mixture was subjected to 20 irradiation  
118 cycles using 100 W microwave energy. The maximum temperature and pressure were 150 °C  
119 and 10<sup>6</sup> Pa respectively. After irradiation, H<sub>2</sub>O (10 ml) was added and the reaction mixture  
120 was centrifuged at 1,400 g for 15 min. An aliquot of the aqueous extract was dried under  
121 reduced pressure and derivatized with BSTFA before GC-MS analysis. Another aliquot was  
122 reduced with NaBH<sub>4</sub> (50 mg) for 1 h at room temperature (Albersheim et al., 1967). The  
123 resulting products (alditols) were silylated with BSTFA. These alditols were identified using  
124 GC comparison with trimethylsilyl ethers of standard alditols. The pellet was extracted at  
125 room temperature during 4 h with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g  
126 for 15 min. The organic phase (extract **2**) was dried under reduced pressure and the products  
127 derivatised with HCl/MeOH and BSTFA. Aliquots of the dried residue **2** (ca. 3 mg) were  
128 analysed using CuPy/GC-MS and CuTHM/GC-MS.

129 The remaining residue **2** was subjected to 20 irradiation cycles with 100 W microwave energy  
130 in 2 ml 1 M HCl. The maximum temperature and pressure were 150 °C and 9 10<sup>5</sup> Pa  
131 respectively. After irradiation, H<sub>2</sub>O (10 ml) was added and the reaction mixture was  
132 centrifuged (1,400 g, 15 min). The aqueous extract was dried under reduced pressure,  
133 derivatised with BSTFA and analysed using GC-MS. The pellet was extracted at room

134 temperature during 4 h with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1 v/v; 30 ml) and centrifuged (1,400 g, 15  
135 min). The extract **3** was dried under reduced pressure and the products derivatised with  
136 HCl/MeOH and BSTFA. Aliquots of the residue **3** were analysed using CuPy/GC-MS and  
137 CuTHM/GC-MS. The residue **3** was subjected to 20 irradiation cycles with 100 W microwave  
138 energy in 2 ml 0.1 M KOH. The maximum temperature and pressure were 150 °C and 6 10<sup>5</sup>  
139 Pa respectively. After irradiation, the reaction mixture was acidified with 1 M HCl and  
140 centrifuged at 1400 g for 15 min. The pellet was extracted at room temperature during 4 h  
141 with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1 v/v; 30 ml) and centrifuged at 1,400 g for 15 min. The supernatant  
142 (extract **4**) was dried under reduced pressure and the products derivatised with HCl/MeOH  
143 and BSTFA. The residue **4** was subjected to 20 irradiation cycles with 100 W microwave  
144 energy in 1 M KOH. The maximum temperature and pressure were 150 °C and 5 10<sup>5</sup> Pa  
145 respectively. The reaction mixture was acidified with 1 M HCl and centrifuged at 1,400 g for  
146 15 min. The pellet was extracted at room temperature during 4 h with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1 v/v;  
147 30 ml) and centrifuged (1,400 g, 15 min). The supernatant (extract **5**) was dried under reduced  
148 pressure and the products derivatised with HCl/MeOH and BSTFA. The residue **5** was  
149 analysed using CuPy/GC-MS and CuTHM/GC-MS. Yields were determined by weighting the  
150 extracts and residues.

151

### 152 2.3. Analytical techniques

153

154 Gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent  
155 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer with electron  
156 ionization at 70 eV. Separation was achieved using a fused silica column coated with DB-  
157 5MS (30 m, i.d. 0.25 mm, film thickness 0.5 µm) with He as carrier gas. The GC oven was  
158 programmed from 100 °C to 320 °C at 4 °C min<sup>-1</sup>. Compound identification was based on the  
159 NIST mass spectrum library or interpretation of the spectra and comparison of GC retention  
160 times with those of standards. When determined, the relative abundances of the positional  
161 isomers of mid-chain hydroxyl alkanolic acids and mid-chain alkanedioic acids (compounds  
162 **23**, **24**, **26**, **27** and **29** in Table 1) were estimated using the mass spectral  $\alpha$ -fragmentation at  
163 the secondary hydroxy group yielding the most intense fragment ion  $m/z$   
164 [MeOOC(CH<sub>2</sub>)<sub>n</sub>CHOTMSi]<sup>+</sup> and assuming that the sensitivity was the same for each isomer.

165

### 166 2.4. Pyrolytic studies

167

168 Curie point pyrolysis gas chromatography-mass spectrometry (CuPy/GC-MS) was performed  
169 using a Pilodist Curie point pyrolyzer. Samples (ca. 1 mg) were pyrolysed for 10 s using  
170 ferromagnetic wires with a Curie temperature of 610 °C under a He flow of 5 ml min<sup>-1</sup>. The  
171 pyrolysis unit was directly coupled to the GC-MS system. The pyrolysis products were  
172 separated using a Thermo Trace GC Ultra gas chromatograph equipped with a 30 m Rtx-5Sil  
173 MS column (0.25 mm i.d., 0.5 µm film thickness). The oven temperature was held at 50 °C  
174 for 10 min and raised to 300 °C at 2°C min<sup>-1</sup>. The gas chromatograph was coupled to a thermo  
175 DSQ mass spectrometer operating at 70 eV. For Curie point thermally assisted hydrolysis and  
176 methylation (CuTHM) with tetramethylammonium hydroxide (TMAH), the same GC-MS  
177 conditions as above were used. Samples (ca. 1 mg) were mixed with 100µL TMAH (25%  
178 w/w in H<sub>2</sub>O), partially dried under reduced pressure and loaded on the ferromagnetic wire  
179 (Curie temperature 610 °C).

180

### 181 **3. Results**

182

#### 183 *3.1. Microwave irradiation conditions*

184

185 The microwave reactor operated up to  $2 \times 10^6$  Pa. In the case of higher pressures the system  
186 was automatically vented. In the closed vessels used in this study, the maximum allowed  
187 pressure corresponded to maximum temperatures of ca. 220 °C and 200 °C in H<sub>2</sub>O and 0.1 M  
188 HCl respectively. Preliminary experiments on humic acids (unpublished results) showed that,  
189 upon continuous microwave irradiation in H<sub>2</sub>O at 220 °C for 30 min, the temperature  
190 (corresponding to a pressure of ca.  $1.7 \cdot 10^6$  Pa) was rapidly reached (< 1 min). When the  
191 temperature was reached the microwave energy strongly decreased (ca. 5-20 W). A two fold  
192 increase in the irradiation time did not improve the yield of extraction. Conversely, the use of  
193 several periods of irradiation followed by a cooling phase allowed to keep a high microwave  
194 energy during a longer time and increased extraction yield (ca. 10% increase in the case of the  
195 studied humic acids). Although we were aware that optimum irradiation energy, temperature  
196 and numbers of cycles depended on the sample matrix, optimisation study was not carried out  
197 for the ROM sample studied owing to the small available amounts of this sample. In this  
198 study we used cycles consisting of irradiation at 100 W for 40 s followed by a cooling phase  
199 of 1 min.

200

#### 201 *3.2. Microwave irradiation in H<sub>2</sub>O*

202  
203 The microwave assisted H<sub>2</sub>O extraction yielded very low amounts of products (1-2% of the  
204 initial ROM). These products (Fig. 2a and Table 1 for peak annotations) were largely  
205 dominated by monosaccharides. Small amounts of disaccharides were also detected at longer  
206 retention times. Apart from predominant sugars, the extract afforded two sets of compounds.  
207 The first one contained aliphatic compounds. It included (i) *n*-alkanoic acids ranging from C<sub>14</sub>  
208 to C<sub>26</sub> with C<sub>16</sub> and C<sub>18</sub> as the major components. A C<sub>18</sub> monounsaturated acid was also  
209 present in the extract. (ii) *n*-alkan-1-ols ranging from C<sub>16</sub> to C<sub>26</sub> and dominated by C<sub>18</sub> and  
210 C<sub>24</sub>, (iii) ω-hydroxy acids ranging from C<sub>16</sub> to C<sub>26</sub> with C<sub>16</sub> and C<sub>22</sub> as major components and  
211 mid-chain hydroxy acids consisting of 8/9/10-hydroxy octadecanoic acid (**23**), 8/9/10,16-  
212 dihydroxy hexadecanoic acid (**27**) and 9,10-dihydroxy octadecanoic acid (**28**). 8-, 9- and 10-  
213 Hydroxy octadecanoic acids accounted respectively for ca. 10, 45 and 45% of the total  
214 isomeric mixture **23** (see 2.4 of Materials and Methods). 10,16- Dihydroxy hexadecanoic acid  
215 was the major isomer (ca. 60% of the total isomeric mixture **27**) followed by 9,16- and 8,16-  
216 dihydroxy hexadecanoic acids (ca. 30 and 10% of the isomeric mixture respectively). The  
217 second set contained aromatic compounds consisting of substituted phenols (**4**, **9**, **12**; Table  
218 1), substituted 2-methoxyphenols (**7**, **14**, **15**, **16**) and substituted 2,6-dimethoxyphenol (**13**).  
219 Based on their mass spectra (Fig. 3), compounds **14** and **15** were considered to belong to the  
220 the guaicyl units. The relative abundances of *p*-hydroxyphenyl, guaiacyl and syringyl units  
221 were estimated using the peak areas in the mass chromatogram, assuming a similar sensitivity  
222 for each compound. Guaiacyl units dominated the aromatics (ca. 45% of the total aromatics  
223 including ca. 4% ferulic acid) followed by *p*-hydroxyphenyl (ca. 20% of the total aromatics  
224 including 11% *p*-coumaric acid) and syringyl units (ca. 11% of the total aromatics).

225

### 226 3.3. Microwave irradiation in HCl

227

228 We could not undoubtedly state that products released using microwave irradiation in aqueous  
229 HCl originated from hydrolysis rather than from an extraction process. However, comparison  
230 of the product distributions between microwave irradiation in HCl and in H<sub>2</sub>O (see  
231 Discussion) suggested that a proportion of the products released from microwave irradiation  
232 in HCl likely originated from acid hydrolysis of some constituents of the ROM. Therefore, in  
233 this paper, we termed microwave irradiation in HCl as microwave HCl hydrolysis, keeping in  
234 mind that this term likely grouped hydrolysis and extraction together.

235



### 236 3.3.1. Microwave irradiation in 0.1 M HCl

237

238 The products released upon microwave irradiation in HCl 0.1 M accounted for ca. 7% of the  
239 residue **1** remaining after microwave irradiation in H<sub>2</sub>O and consisted mainly of  
240 monosaccharides. The aqueous extract (Fig. 1) accounted for ca. 70% of the products released  
241 upon microwave irradiation in 0.1 M HCl and consisted predominantly of monosaccharides.  
242 Low abundances of disaccharides and trace amounts of amino acids (mainly aspartic acid and  
243 5-oxoproline) were also detected through GC-MS. Analysis of the trimethylsilyl ethers of the  
244 alditols of the aqueous extract revealed that glucose was by far the predominant  
245 monosaccharide present in the aqueous extract from microwave 0.1 M HCl hydrolysis.  
246 Xylose and mannose were observed at extremely low levels. Arabinose and galactose were  
247 not found.

248 The organic extract (extract **2**) was also largely dominated by monosaccharides (Fig 2b),  
249 glucose being the major one based on retention times. Apart from the largely dominant  
250 glucose, the organic extract from microwave 0.1 M HCl hydrolysis gave two additional  
251 compound classes (Fig. 2b and Table 1 for peak annotations). The first one consisted of  
252 aliphatic compounds and included (i) *n*-alkanoic acids ranging from C<sub>14</sub> to C<sub>32</sub> with C<sub>16</sub> and  
253 C<sub>18</sub> as major components. A C<sub>18</sub> monounsaturated acid was also detected, (ii) *n*-alkan-1-ols  
254 from C<sub>12</sub> to C<sub>32</sub> with C<sub>22</sub> and C<sub>24</sub> as major components, (iii)  $\omega$ -hydroxy alkanolic acids ranging  
255 from C<sub>16</sub> to C<sub>28</sub> and dominated by C<sub>22</sub>, (iv) mid-chain hydroxy alkanolic acids comprising  
256 8/9/10-hydroxy octadecanoic acid (each isomer accounted respectively for ca. 10, 45 and 45%  
257 of the isomeric mixture **23**), 8/9/10,16-dihydroxy hexadecanoic acid (each isomer accounted  
258 respectively for ca. 10, 30 and 60% of the isomeric mixture **27**) and 7/8-hydroxy  
259 hexadecanedioic (**26**). The relative abundances of these isomers were respectively ca. 40 and  
260 60% of the isomeric mixture **26**.  $\alpha,\omega$ -Alkanedioic acids were also detected in trace amounts.  
261 The second class contained aromatic compounds comprising substituted phenols (**1**, **2**, **3**, **4**, **9**,  
262 **12**), substituted 2-methoxyphenols (**6**, **7**, **16**) and substituted 2,6-dimethoxy phenols (**13**). On  
263 the basis of their mass spectra tentatively identified and unidentified compounds (**8**, **14**, **15**,  
264 **17**, **18** and **19**, Fig. 3) were considered to belong to this aromatic class. This class was largely  
265 dominated by guaiacyl units (ca. 57% of the total aromatics including 1% ferulic acid). *p*-  
266 Hydroxy phenols (including 2% *p*-coumaric acid) and syringyl units accounted for ca. 8 and  
267 16% of the total aromatics, respectively. Isomeric cyclodimers of *p*-coumaric acid (**20**) were  
268 also detected in small amounts. The mass spectra of isomers **20** (as methyl ester,  
269 trimethylsilyl ether derivatives) were similar and exhibited the same major ions (*m/z* 250,

270 235) as in the mass spectrum of the methyl ester, trimethylsilyl ether of *p*-coumaric acid and  
271 their fragmentation patterns (Fig.3g) were consistent with cyclodimers of *p*-coumaric acid  
272 (Ford and Hartley, 1989).

273

### 274 3.3.2. Microwave irradiation in 1 M HCl

275

276 The products released upon microwave irradiation in 1 M HCl accounted for ca. 6% of the  
277 residue **2** remaining after microwave irradiation in 0.1 M HCl. The aqueous extract (ca. 60%  
278 of the products released upon microwave irradiation) and the organic extract (extract **3**) were  
279 qualitatively the same as those resulting from microwave irradiation in 0.1 M HCl. Glucose  
280 largely dominated both aqueous and organic extracts. In the aqueous extract, disaccharides  
281 were hardly detected and trace amounts of the same amino acids as in the aqueous extract  
282 from microwave irradiation in 0.1 M HCl were present (data not shown). Similar aromatic  
283 and aliphatic compounds were identified in the organic extract **3** (data not shown). However,  
284 the relative abundances of the aromatic and aliphatic compounds were much lower than in the  
285 organic extract from the microwave irradiation in 0.1 M HCl.

286

### 287 3.4. Microwave irradiation in KOH

288

289 As in the case of microwave irradiation in HCl, it could be assumed that hydrolysis  
290 (particularly of esters) occurred upon microwave irradiation in KOH. Microwave irradiation  
291 of the residue **3** in 0.1 M KOH yielded very low amounts of products. The organic extract  
292 (extract **4**) accounted for ca. 1% of the residue. By contrast, the organic extract from  
293 microwave irradiation in 1 M KOH (extract **5**) accounted for 22% of the residue **4**. Similar  
294 products were identified in both extracts, so only the products released upon microwave  
295 irradiation in 1 M KOH were presented here. Fig. 2c depicted the distribution of the products  
296 extracted from the microwave 1 M KOH hydrolysate (extract **5**). The microwave KOH  
297 hydrolysis yielded two main compound classes. The first contained aliphatic compounds  
298 which largely dominated the extract. It consisted in (i) *n*-alkanoic acids ranging from C<sub>14</sub> to  
299 C<sub>32</sub> with C<sub>16</sub>, C<sub>18</sub> and C<sub>28</sub> as the major constituents. A C<sub>18</sub> monounsaturated acid was also  
300 present, (ii) *n*-alkan-1-ols from C<sub>16</sub> to C<sub>32</sub> with maximum at C<sub>22</sub> and C<sub>24</sub>, (iii) ω-hydroxy  
301 alkanolic acids ranging from C<sub>14</sub> to C<sub>28</sub> and maximising at C<sub>22</sub>. A monounsaturated C<sub>18</sub> ω-  
302 hydroxy acid was also detected in low amounts, (iv) mid-chain hydroxy alkanolic acids (**21**,  
303 **22**, **23**, **25**, **27**, **28**, **29**, **30**, Table 1 for peak annotations) dominated by 8/9/10,16 dihydroxy

304 hexadecanoic acids (each isomer accounted respectively for ca 10, 30 and 60% of the  
305 isomeric mixture **27**) and hydroxy alkanedioic acids (**24**, **26**) comprising C<sub>15</sub> and C<sub>16</sub>  
306 homologues with 7/8-hydroxy hexadecanedioic (each isomer accounted for ca. 40 and 60% of  
307 the isomeric mixture **26**) as the major component.  $\alpha,\omega$ -Alkanedioic acids ranging from C<sub>16</sub> to  
308 C<sub>28</sub> with C<sub>22</sub> and C<sub>24</sub> as the major constituents were also detected in trace amounts. The  
309 second class comprised aromatic compounds consisting of substituted phenols (**1**, **2**, **3**, **4**, **9**,  
310 **10**, **12**, Table 1 for peak annotations), substituted 2-methoxyphenols (**5**, **6**, **7**, **14**, **16**) and  
311 substituted 2,6-dimethoxyphenols (**13**). Isomeric cyclodimers of *p*-coumaric acid (**20**) were  
312 also present in substantial amount. This compound class was dominated by guaiacyl units (ca.  
313 46% of the total aromatics, including 9% ferulic acid) and *p*-hydroxy phenols (ca. 38% of the  
314 total aromatics, including 29% *p*-coumaric acid). Syringic units accounted for ca. 6% of the  
315 total aromatics.

316

### 317 3.5. Pyrolytic study of the residues

318

319 In order to substantiate the efficiency and specificity of the microwave irradiation under  
320 acidic and basic conditions, the insoluble residues obtained after 0.1, 1 M HCl and 1 M KOH  
321 microwave hydrolyses (residues **2**, **3** and **5**) were subjected to Curie point pyrolysis  
322 (CuPy/GC-MS) and thermally assisted hydrolysis and methylation (CuTHM/GC-MS).  
323 Microwave irradiation in H<sub>2</sub>O and in 0.1 M KOH releasing very low amounts of products,  
324 therefore, the CuPy/GC-MS and CuTHM/GC-MS of the corresponding residues were not  
325 performed.

326

#### 327 3.5.1. CuPy/GC-MS

328

329 CuPy/GC-MS traces for the ROM and for the residues **2**, **3** and **5** were shown in Fig. 4. The  
330 identified products were listed in Table 2. The ROM pyrolysate (Fig. 4a) was characterised by  
331 the presence of four main compound classes. The first was constituted of furans (**1**, **2b**, **8**),  
332 levoglucosenone (**6**) and levoglucosan (**15**) which were generally considered as  
333 polysaccharide pyrolysis products. Aromatic compounds (**2a**, **3**, **4**, **5**, **7**, **9-14**, **16**), likely  
334 related to lignin, constituted the second class. The third class was composed of *n*-alkane/*n*-  
335 alkene doublets. The series, ranging from C<sub>9</sub> to C<sub>32</sub> did not show any clear carbon number  
336 predominance, except the relatively high abundance of the C<sub>20</sub>, C<sub>22</sub> and C<sub>24</sub> homologues. The

337 last class was constituted by *n*-alkan-2-one/*n*-alken-2-one doublets. The series, with a strong  
338 odd/even predominance, ranged from C<sub>19</sub> to C<sub>35</sub> with C<sub>29</sub> and C<sub>27</sub> as the major components.

339 The pyrochromatogram of the residue obtained after microwave 0.1 M HCl hydrolysis (Fig.  
340 4b; Table 2 for peak annotations) shown a strong decrease in the relative abundance of  
341 levoglucosan (**15**). The decrease in the relative abundances of the other compounds related to  
342 polysaccharides (i.e. **1**, **2b**, **6**, **8**) was more pronounced after microwave 1 M HCl hydrolysis  
343 (Fig. 4c; Table 2 for peak annotations). Within the other classes (aromatic, *n*-alkane/*n*-alkene  
344 and *n*-alkan-2-one/*n*-alken-2-one), the relative abundances of the constituents appeared to  
345 remain rather constant after these irradiations.

346 The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolysis (Fig.  
347 4d) shown a strong decrease of the relative abundances of the *n*-alkane/*n*-alkene and *n*-alkan-  
348 2-one/*n*-alken-2-one doublets. It was dominated by 2-methoxy phenol (**5**) and 4-methyl-2-  
349 methoxyphenol (**7**).

350

### 351 3.5.2. CuTHM/GC-MS

352

353 Fig. 5 shown the total ion current (TIC) chromatograms of the thermochemolysates of the  
354 ROM and of the residues **2**, **3** and **5**. The compounds identified as methyl esters and methyl  
355 ethers were listed in table 3.

356 The CuTHM pyrolysate of the ROM (Fig. 5a) was quite similar to that obtained by Quénéa et  
357 al. (2005a). Two main product classes constituted this pyrolysate. The first was constituted by  
358 aromatic compounds (**4-19**, **27**) which were dominated by the methylated counterparts of  
359 vanillic acid (**14**) and *p*-coumaric acid (**17**). Isomeric cyclodimers of *p*-coumaric acid (**27**)  
360 were also present at longer retention time. The second class contained aliphatic compounds  
361 including as the major constituents: (i) *n*-alkanoic acids (a C<sub>18</sub> monounsaturated acid was  
362 identified), ranging from C<sub>16</sub> to C<sub>32</sub> with a strong even carbon number predominance. The  
363 distribution was dominated by long chain components (>C<sub>20</sub>); (ii) *n*-alkan-1-ols ranging from  
364 C<sub>20</sub> to C<sub>32</sub> with a strong even/odd predominance and a maximum at C<sub>22</sub>. (iii)  $\omega$ -hydroxy acids  
365 ranging from C<sub>16</sub> to C<sub>30</sub> with an even/odd predominance and C<sub>22</sub> and C<sub>24</sub> as the major  
366 components; (iv) long chain  $\alpha,\omega$ -alkanedioic acids, present in lower relative abundances,  
367 ranging from C<sub>20</sub> to C<sub>28</sub>. The distribution exhibited an even/odd predominance and was  
368 dominated by C<sub>24</sub>; (v) mid-chain hydroxy alkanolic and hydroxy alkanedioic acids (**20-26**,  
369 Table 3 for peak annotation). The tentative identification of these compounds was based on  
370 the interpretation of their mass spectra (Fig. 6). When several structures were possible, the

371 carbon chain lengths found for the mid-chain hydroxy compounds identified in the extracts  
372 were selected (i.e. C<sub>15</sub>, C<sub>16</sub> and C<sub>18</sub>). The resulting identification suggested an incomplete  
373 methylation of some hydroxyl groups (compounds **22**, **24** and **26**). A minor series of *n*-  
374 alkane/*n*-alkene doublets ranging from C<sub>11</sub> to C<sub>33</sub> with no obvious carbon number  
375 predominance was also identified.

376 The pyrochromatograms of the residues obtained after microwave HCl hydrolyses shown a  
377 decrease in the relative abundances of the aliphatic compounds (Fig. 4b, c) with the exception  
378 of *n*-alkane/*n*-alkene doublets whose relative abundances appeared to increase. The decrease  
379 in the relative abundances of aliphatic compounds was clearly more pronounced after  
380 microwave KOH hydrolysis (Fig. 4d).

381 The TIC trace for the residue remaining after microwave irradiation in 1 M KOH was largely  
382 dominated by aromatic compounds with vanillic acid (**14**) and *p*-coumaric acid (**17**) as the  
383 major constituents. Within the aliphatic compound class, the decrease in the relative  
384 abundances of  $\omega$ -hydroxy acids resulting from microwave KOH hydrolysis, appeared to be  
385 stronger than that of the other constituents (e.g. alkanolic acids or alkanols).

386

## 387 **4. Discussion**

388

### 389 *4.1. Comparison between microwave irradiation in H<sub>2</sub>O and HCl. Extraction or hydrolysis?*

390

391 Although we cannot completely rule out the possibility of bond cleavage under the influence  
392 of temperature and pressure, we can assume that products released on microwave irradiation  
393 in H<sub>2</sub>O arose mainly from a desorption process. It is then likely that monosaccharides, lignin-  
394 derived and aliphatic compounds found in the H<sub>2</sub>O extract are present as such in the ROM.  
395 These compounds, likely adsorbed on (or entrapped in) the macromolecular structure of the  
396 ROM, were not released during the isolation of the ROM despite the drastic acid hydrolyses  
397 applied (Quénéa et al. 2005a). This could indicate that microwave energy interacts efficiently  
398 with either the macromolecular structure or the adsorbed (or entrapped) compounds and is  
399 able to disrupt such interactions. Contrary to the extract from microwave irradiation in H<sub>2</sub>O  
400 and 0.1 M HCl, disaccharides were hardly detected in the extract from microwave irradiation  
401 in 1 M HCl, suggesting that at least part of the monosaccharides in 1 M HCl extract arises  
402 from an acid hydrolysis of oligo- and/or polysaccharides. The occurrence of acid hydrolysis  
403 during microwave irradiation in HCl is also supported by the presence of minute amounts of  
404 amino acids which were not observed in the extract from microwave irradiation in H<sub>2</sub>O. The

405 predominance of monosaccharides in the extracts from microwave irradiation in H<sub>2</sub>O and HCl  
406 indicates that intact monosaccharides, and probably oligo- and polysaccharides, are still  
407 present in the ROM despite the intensive trifluoroacetic acid and HCl hydrolyses performed  
408 during isolation (Quénéa et al., 2005a).

409 The GC-MS traces revealed a very similar distribution of aliphatic compounds in the extracts  
410 from microwave irradiation in both 0.1 M HCl and H<sub>2</sub>O (Figs. 2a, b). By contrast, some  
411 differences are observed for the aromatic compound distributions between these extracts. The  
412 greatest difference is a greater range of aromatic compounds in the extract from microwave  
413 HCl hydrolysis. Seventeen aromatic compounds and cyclodimers of *p*-coumaric acid were  
414 identified in the extract from microwave irradiation in 0.1 M HCl while only eight aromatic  
415 compounds were present in the extract from microwave irradiation in H<sub>2</sub>O. This difference,  
416 together with the strong decrease in the relative abundances of disaccharides and the presence  
417 of amino acids in the extracts from microwave HCl hydrolyses, suggest that hydrolysis of  
418 some constituents of the ROM, in this case, carbohydrate, protein and lignin moieties,  
419 occurred upon microwave irradiation in HCl.

420

#### 421 4.2. Origin of aliphatic compounds

422

423 Aliphatic compounds in all the extracts from microwave irradiation in H<sub>2</sub>O, HCl and KOH,  
424 are well known constituents of cutin and suberin. The polyester cutin, present in the majority  
425 of the aerial parts of vascular plants, and suberin, present in the bark and roots of vascular  
426 plants, differ in the chain length and the substitution patterns of their monomers (Walton,  
427 1990; Kolattukudy, 2001). Long chain ( $\geq C_{20}$ ) *n*-alkanedioic acids,  $\omega$ -hydroxy acids, *n*-  
428 alkanolic acids and, to a lesser extent, *n*-alkan-1-ols are frequently dominant monomers of  
429 suberin, while mid-chain substituted monomers are usually minor constituents. On the  
430 contrary, cutin is characterised by substantial abundances of C<sub>16</sub> and C<sub>18</sub> mid-chain  
431 substituted monomers, while long chain monomers ( $\geq C_{20}$ ) are rarely present. In the extracts  
432 from microwave irradiation in H<sub>2</sub>O and in HCl (Figs. 2a, b), the distribution patterns of  
433 aliphatic compounds are quite similar. Apart from ubiquitous C<sub>16</sub> and C<sub>18</sub> *n*-alkanoic acids,  
434 the aliphatic compounds are dominated by long chain ( $\geq C_{20}$ ) components which can be  
435 considered as predominantly from a suberin source. However, the high relative abundances of  
436 C<sub>22</sub> and C<sub>24</sub> *n*-alkan-1-ols suggests they could also originate from the plant wax esters in

437 which they are widespread (Bianchi, 1995). The mid-chain hydroxy constituents, probably  
438 originating from cutin, are present in lower amounts.

439 Microwave irradiation in KOH is more efficient for promoting hydrolysis of ester linkages of  
440 cutin and suberin than microwave irradiation in HCl. Therefore, the distribution pattern of  
441 aliphatic compounds in the extract from 1 M KOH hydrolysis (Fig. 2c) strongly differs from  
442 that of aliphatic compounds in the extracts from microwave irradiation in HCl (Fig. 2b).  $\omega$ -  
443 Hydroxy alkanolic acids with chain length  $\geq C_{20}$ , likely originating from suberin, largely  
444 dominate. In addition, this extract contains  $C_{15}$ ,  $C_{16}$  and  $C_{18}$  mid-chain hydroxy alkanolic and  
445  $C_{15}$  and  $C_{16}$  mid-chain alkanedioic acids in high amounts. This indicates a significant  
446 contribution of cutin to the ROM. The contribution of cutin from pine, the predominant  
447 vegetation, is indicated by the presence of trace amounts, of  $C_{12}$  and  $C_{14}$   $\omega$ -hydroxy alkanolic  
448 acids, which have been reported as typical constituents of cutin of the needles of numerous  
449 species of gymnosperms (Matzke and Riederer, 1991; Nierop, 2001; Nierop and Verstraten,  
450 2004; Otto and Simpson, 2006). The contribution of cutin from pine needles could be also  
451 suggested by the presence of mid-chain hydroxy pentadecanoic acids (**21**, **22**, **25**; Table 1)  
452 and pentadecanedioic acid (**24**; Table 1), which have been reported as monomers of cutin of  
453 some gymnosperms (Hunneman and Eglinton, 1972), pine needles and grasses (Otto and  
454 Simpson, 2006). The dominant contribution of 8,16- and 10,16-dihydroxy hexadecanoic acid  
455 to the dihydroxy hexadecanoic isomeric mixture (respectively ca. 30 and 60% of the isomeric  
456 mixture **27** in all the extracts) suggests a significant contribution of cutin from the  
457 undergrowth (predominantly grasses and ferns). Indeed according to Goñi and Hedges (1990),  
458 in contrast to most of the gymnosperm species, Poaceae species produced high yield of cutin-  
459 derived 8,16- and 10,16-dihydroxy hexadecanoic acids. Moreover, 10,16-dihydroxy  
460 hexadecanoic acid has been reported as the most abundant component in the solvent-extracted  
461 soil under grasses and ferns (Naafs and van Bergen, 2002) The relative contributions of  
462 suberin and cutin to the ROM may be estimated using monomers typical of cutin or suberin  
463 and monomers common to both polyesters as suggested by Otto and Simpson (2006). The  
464 suberin/ cutin ratio of 1.8 calculated in this way from the microwave 1 M KOH hydrolysis for  
465 the ROM corroborates the predominance of suberin input previously reported (Quénéa et al.,  
466 2005a). According to Otto and Simpson (2006), this value resembles that obtained for  
467 grassland soils rather for pine forest ones for which values  $< 1$  were found. This could  
468 indicate an important contribution of suberin from the undergrowth in addition to that of the  
469 cutin previously suggested.

470

471 *4.3. Origin of aromatic compounds*

472

473 Apart from the tentatively identified (**14**, **15**) and unidentified (**17-19**) aromatic compounds  
474 which were mainly found in the extract from microwave irradiation in 0.1 M HCl (Fig. 2b),  
475 similar aromatic compounds were present in all the extracts (Fig. 2; Table 1). These  
476 compounds were predominantly guaiacyl, *p*-hydroxy and syringyl phenols and were generally  
477 considered to be derived from lignin (eg. Brunow, 2001). However, substituted  
478 dihydroxyphenols (e.g. **9**, detected in significant amounts in the extract from microwave  
479 irradiation in H<sub>2</sub>O and 0.1 M HCl, and **10**, present in the extract from microwave irradiation  
480 in 1 M KOH) could also originate from (condensed) tannins (e.g. Galletti et al., 1995; Nierop  
481 et al., 2005). *p*-Coumaric (**12**), ferulic (**16**) acids and 4-hydroxybenzaldehyde (**1**) can also  
482 arise from the aromatic domain of suberin (Walton, 1990; Bernards, 2002) to which they  
483 could be linked via ester bonds (Bernards and Lewis, 1998). As in the case of  
484 monosaccharides, the presence of these aromatic compounds in the extract from microwave  
485 irradiation in H<sub>2</sub>O suggests that lignin monomers were present as such in the ROM. They  
486 could be adsorbed on (or entrapped in) the macromolecular structure of the ROM or they  
487 could form molecular associations with carbohydrates and could not be released upon  
488 extractions and hydrolyses of the isolation process. The relative abundances of guaiacyl and  
489 syringyl units increase upon microwave irradiation in 0.1 M HCl. The units released upon  
490 these conditions are likely present as arylglycerol- $\beta$ -aryl ether structures, such structures  
491 being acid-hydrolysable (Adler et al., 1957; Lundquist and Lundgren, 1972). The tentative  
492 structures attributed to compounds **8**, **14**, **15**, **17**, **19** present in the extract from microwave  
493 irradiation in 0.1 M HCl (Fig. 3), could support this suggestion. Indeed similar types of  
494 ketones were found upon acid degradation of lignin (Adler et al., 1957; Lundquist and  
495 Lundgren, 1972). On the other hand, microwave irradiation in KOH leads to an increase in the  
496 relative abundances of *p*-coumaric, and ferulic acids. This suggests that these acids were  
497 predominantly in an ester-linked form in the ROM. They could be present esterified to lignin-  
498 polysaccharide complex or in the aromatic domain of suberin (e.g. Kolattukudy, 2001). The  
499 predominance of the guaiacyl over the syringyl units in all the extracts is consistent with the  
500 dominant gymnosperm vegetation. However, the significant amounts of *p*-hydroxyphenyl  
501 units, including high amount of *p*-coumaric acid, and ferulic acid, particularly in the extract  
502 from microwave irradiation in KOH, suggests a significant non-woody angiosperm  
503 undergrowth (likely gramineous plants) influence.



504 Interestingly is the presence of cyclodimers of *p*-coumaric acid (**20**) in the extract from  
505 microwave HCl and KOH hydrolyses. To our knowledge, these aromatic compounds have  
506 never been reported in soil lipids or in degradation products of soils or non-hydrolysable OM  
507 from soils. Cyclodimers of *p*-coumaric (or ferulic) acid have been reported as constituents of  
508 gramineous plant cell walls (Krauze-Baranowska, 2002 and references therein). They are  
509 thought to be synthesized by photodimerisation of *p*-coumaric acid and to reduce the  
510 biodegradability of the cell walls (Ford and Hartley, 1989). Their presence supports a  
511 contribution of gramineous-derived compounds to the ROM although cyclodimerisation of *p*-  
512 coumaric acid under microwave irradiation cannot be completely disregarded.

513

#### 514 *4.4. Pyrolysis of the residues. Comparison of microwave extractions/hydrolyses with pyrolysis*

515

516 In good agreement with earlier pyrolytic studies of the same ROM sample (Quénéa et al.,  
517 2005a), the pyrochromatogram obtained by CuPy/GC-MS of the ROM (Fig. 4a) is dominated  
518 by products commonly observed for the pyrolysis of cellulose and related carbohydrates  
519 (Pouwels et al., 1989; Pastorova et al., 1994, Gauthier et al., 2003) i.e. levoglucosan,  
520 levoglucosenone and 2,3-dihydrobenzofuran. A series of *n*-alkane/*n*-alkene doublets is  
521 present in high relative abundance. Two main origins are generally considered for these  
522 doublets, i.e. non-hydrolysable macromolecules of higher plants such as cutan or suberan  
523 (Tegelaar et al., 1989; 1995; Augris et al., 1998; Nierop, 1998) or lipids incorporated through  
524 covalent bonds into the recalcitrant macromolecular structures (Almendros and Sanz, 1992;  
525 Almendros et al., 1996). The distribution pattern of this series appears to result from a  
526 combination of a smooth distribution centered between C<sub>24</sub> and C<sub>27</sub> and separate C<sub>20</sub>, C<sub>22</sub> and  
527 C<sub>24</sub> doublets. This suggests that the *n*-alkane/*n*-alkene doublets likely derive from the two  
528 aforementioned origins, the dominant C<sub>20</sub>, C<sub>22</sub> and C<sub>24</sub> doublets likely originating from  
529 incorporated lipids. The series of *n*-alkan-2-ones observed here, was not detected by Quénéa  
530 et al. (2005a) in the pyrolysate of the same ROM sample, while it was observed, with a  
531 similar distribution, in the pyrochromatogram of the double-shot pyrolysis of the same ROM  
532 sample (Quénéa et al., 2006b). The differences in the techniques (e.g. magnetic wire vs  
533 magnetic tubes) and/or temperature (610 °C in the present study, 650 °C for Curie point  
534 pyrolysis and 600 °C for the double-shot pyrolysis) could explain these discrepancies. The  
535 origin of *n*-alkan-2-ones is still a subject of speculation. β -Oxidation of *n*-alkanes (e.g.  
536 Cranwell et al., 1987; Jaffé et al., 1996), β-oxidation and subsequent decarboxylation of *n*-  
537 alkanolic acids (Amblès et al., 1989) and direct inputs of *n*-alkan-2-ones naturally occurring in

538 living organisms (e.g. Volkman et al., 1983; Quéneá et al. 2006b) have been postulated as  
539 possible origins for *n*-alkan-2-ones present in sediments, soils or recalcitrant organic matter.  
540 Moreover, as far as we are aware, such ketone doublets have not been previously reported in  
541 pyrolysates. Their presence would indicate that the keto group is not formed upon pyrolysis,  
542 the doublets being formed in a similar way as the *n*-alkane/*n*-alkene doublets.

543 Several phenolic compounds generally considered to originate from pyrolysis of lignin (e.g.  
544 Gauthier et al., 2003) were also detected in high relative abundances (Fig. 4a; Table 2). It  
545 should be noted that most of the phenolic compounds detected may be related to guaiacyl  
546 units. Only phenol (**3**, Table 2), which displays a rather low relative abundance, may be  
547 related to *p*-coumaric acid. However the composition of the extracts, especially that from  
548 microwave 1 M KOH hydrolysis (Fig 2c) indicates a substantial contribution of *p*-coumaric  
549 acid to the ROM. In addition, the methylated form of *p*-coumaric acid is present as one of the  
550 major aromatic compounds in the CuTHM pyrolysate of the ROM (Fig. 5; Table 3). This  
551 indicates that CuPy technique likely underestimates the contribution of *p*-coumaric acid to the  
552 ROM.

553 The strong decrease in the relative abundances of the compounds derived from  
554 polysaccharides (**1**, **2b**, **6**, **8**, **15**; Table 2) in residues from the microwave HCl hydrolyses  
555 (Figs. 4b, c) is consistent with the predominance of monosaccharides in the extracts from  
556 microwave HCl hydrolyses. In spite of drastic acid hydrolyses and extractions applied for the  
557 ROM isolation, mono- and oligosaccharides are still present in significant amounts in the  
558 ROM. The presence of such compounds in the ROM could be explained by the fact that  
559 mono- and oligosaccharides belong to molecular associations (e.g. lignin-carbohydrate  
560 complex) which could not be disrupted by the conventional heating used during the isolation  
561 process. However, these molecular associations containing highly polar carbohydrates could  
562 be disrupted upon microwave irradiation. Therefore it appears that carbohydrates could be  
563 released from covalent or physicochemical entrapment. However, although greatly reduced,  
564 the polysaccharidic contribution to the ROM was not completely removed, even after  
565 microwave irradiation in 1 M HCl (Fig. 4c). The microwave HCl hydrolyses do not result in a  
566 noticeable decrease in the relative abundance of compounds considered to derive from lignin  
567 (i.e. **2a**, **3**, **5**, **7**, **9-14**, **16**; Table 2). Similar trend is observed for the *n*-alkane/*n*-alkene and *n*-  
568 alkanon-2-one/*n*-alken-2-one doublets. This is consistent with the low relative abundances of  
569 aromatic and aliphatic compounds present in the extract from microwave irradiation in HCl  
570 (Fig. 2b).

571 The pyrochromatogram of the residue obtained after microwave 1 M KOH hydrolyses reveals  
572 a strong decrease in the relative abundances of *n*-alkane/*n*-alkene and *n*-alkan-2-one/*n*-alken-  
573 2-one doublets (Fig. 4d). Furthermore, among the series of *n*-alkane/*n*-alkene doublets, the  
574 relative abundances of C<sub>22</sub> and C<sub>24</sub> homologues (assumed to derive from covalent linked  
575 lipids) tend to decrease and fall in the same range as the whole series. This could be correlated  
576 to the high relative abundances of C<sub>22</sub> and C<sub>24</sub> *n*-alkan-1-ols and/or ω-hydroxy alkanolic acids  
577 observed in the extract from microwave 1 M KOH hydrolysis (Fig 2c) and in the CuTHM  
578 pyrolysates of the ROM and the residues obtained after microwave HCl hydrolyses (Figs. 5a,  
579 b, c). This suggests that at least part of the predominant C<sub>22</sub> and C<sub>24</sub> *n*-alkane/*n*-alkene  
580 doublets originate from such C<sub>22</sub> and C<sub>24</sub> alcohols and/or ω-hydroxy alkanolic acids (or from  
581 the moieties (eg. esters) from which they derive).

582 The microwave irradiation in 1 M KOH results in a substantial decrease in the relative  
583 abundances of phenolic compounds with the exception of 2-methoxyphenol (**5**) and 4-methyl-  
584 2-methoxyphenol (**7**) which largely dominate the pyrochromatogram (Fig. 4d). These two  
585 compounds can be related to guaiacyl units of lignin

586 The distribution pattern of the compounds released upon CuTHM of the ROM (Fig. 5a) is in  
587 good agreement with that obtained by Quénéa et al. (2005a) for the same sample. The  
588 pyrochromatogram is dominated by aliphatic and aromatic compounds considered to derive  
589 respectively from cutin and/or suberin and lignin. However, notable differences between the  
590 two distributions are observed. *n*-Alkan-1-ols, mid-chain hydroxy alkanolic acids and  
591 cyclodimers of *p*-coumaric acid observed in the present CuTHM pyrolysate have not been  
592 identified by Quénéa et al. (2005a). Mid-chain hydroxy alkanolic acids (**20-26**; Table 3) were  
593 tentatively identified on the basis of their mass fragmentation and by selecting the carbon  
594 chain lengths found for this type of compounds in the extracts from the microwave  
595 irradiations (ie C<sub>15</sub>, C<sub>16</sub> and C<sub>18</sub>). The proposed structures (Fig.6) involve the presence of non-  
596 methylated hydroxyl groups in some of these compounds. Providing that these structures are  
597 correct, this indicates that hydroxyl groups are only partially methylated upon CuTHM. The  
598 partial methylation of hydroxyl groups have been reported in earlier studies (de Leeuw and  
599 Baas, 1993, Kralert et al., 1995). It has been suggested that partial methylation of alkanols  
600 upon CuTHM is due to competition between methylation and thermovaporization of these  
601 compounds (de Leeuw and Baas, 1993, Kralert et al., 1995). The aromatic compounds are  
602 dominated by the methylated counterparts of vanillic (**14**) and *p*-coumaric (**17**) acids (Fig. 5a;  
603 Table 3).

604 Contrary to CuPy, typical products related to carbohydrates are not detected in the CuTHM  
605 pyrolysate of the ROM. This is consistent with previous observations (Quénéa et al., 2005a).  
606 It must also be noted that the major aromatic products from the CuPy (**5** and **7**; Fig. 4) appear  
607 in CuTHM pyrolysate as rather minor products (**4** and **6**; Fig 5) whereas vanillic (**14**) and *p*-  
608 coumaric (**17**) acids largely dominate. This highlights the much higher efficiency of CuTHM  
609 when compared to CuPy for the detection of lignin-derived compounds.

610 The CuTHM pyrolysates of the residues obtained after microwave HCl hydrolyses (Figs. 5b,  
611 c) reveal a decrease in the relative abundances of aliphatic compounds. By contrast, and as  
612 already noticed for the CuPy pyrolysates of these residues, no notable change in the relative  
613 abundances of aromatic compound is observed. The decrease in the relative abundances of  $\omega$ -  
614 hydroxy acids and, to a lesser extent that of *n*-alkan-1-ols, appears to be more pronounced  
615 than that of *n*-alkanoic acids. For example, when the CuTHM pyrolysate of the ROM and that  
616 of the residue from microwave 1 M HCl hydrolysis are compared (Fig. 5a, c), the C<sub>22</sub>  $\omega$ -  
617 hydroxy acid to C<sub>22</sub> *n*-alkanoic acid and C<sub>22</sub> *n*-alkan-1-ol to C<sub>22</sub> *n*-alkanoic acid ratios are  
618 respectively ca. 3 times and 2 times lower in the latter pyrolysate, while the C<sub>22</sub>  $\omega$ -hydroxy  
619 acid to C<sub>22</sub> *n*-alkan-1-ol ratio is only 1.3 times lower. The same trend is observed for C<sub>24</sub>  
620 homologues (Table 4). It appears that *n*-alkanoic acids are more difficult to release by  
621 microwave HCl hydrolyses. This is consistent with the distribution of the aliphatic  
622 compounds in the extract from microwave HCl hydrolyses. Indeed, in these extracts, the  
623 aliphatic compounds were dominated by  $\omega$ -hydroxy acids and *n*-alkan-1-ols. This result  
624 suggests the existence of different pools for *n*-alkanoic acids on the one hand, and for  $\omega$ -  
625 hydroxy acids and *n*-alkan-1-ols on the other hand.

626 In good agreement with the composition of the extract from microwave 1M KOH hydrolysis,  
627 the decrease in the relative abundances of aliphatic compounds is especially marked in the  
628 CuTHM pyrolysate of the final residue (Fig. 5d). However, in contrast to the CuTHM of the  
629 residues from microwave HCl hydrolyses, the decrease in the relative abundances of the  
630 different aliphatic compounds appears to be uniform (Table 4). Regarding the aromatic  
631 compound class (**1-18**; Figs 5a-d) no noticeable change in the relative abundance of its  
632 constituents were observed between the CuTHM pyrolysates of the ROM and the residues **2**,  
633 **3** and **5**. The final residue obtained after microwave extractions and hydrolyses appears to be  
634 predominantly composed of aromatic compounds with vanillic and *p*-coumaric acids as the  
635 major components. This indicates that at least part of lignin-derived constituents are resistant  
636 to microwave acid and base hydrolyses. By contrast, these constituents can be released by

637 thermal cracking. However, the microwave irradiation of commercial lignin (Lignin,  
638 hydrolytic, Aldrich) in 0.1 M NaOH resulted in almost 100% degradation (unpublished  
639 results). This suggests that lignin in the ROM is efficiently protected.

640 At this stage, work is preliminary and the mechanistic aspects of microwave hydrolyses of  
641 such recalcitrant macromolecular material must be studied and the range of samples  
642 expanded.

643

## 644 **5. Conclusions**

645

646 The recalcitrant organic matter (ROM) isolated from a forest soil was submitted to sequential  
647 microwave assisted extractions and/or hydrolyses in H<sub>2</sub>O, HCl and KOH, resulting in ca. 35%  
648 degradation of the initial ROM. The overall extracts consisted in ca. 10% carbohydrates and  
649 ca. 25% aliphatic and aromatic compounds.

650 The differences between the distributions in the extracts from microwave irradiation in H<sub>2</sub>O  
651 and in HCl suggest that acid hydrolysis of oligo(poly)saccharides and oligo(poly)peptides  
652 occurred upon microwave irradiation in HCl.

653 Products resulting from microwave irradiation in H<sub>2</sub>O and in HCl are strongly dominated by  
654 glucose. The resistance of carbohydrates to the acid hydrolyses and extractions performed  
655 during the isolation of the ROM is thought to originate from the presence of carbohydrate-  
656 containing molecular associations. These molecular associations contain polar functions  
657 suitable for localized superheating effects under microwave irradiations and subsequent  
658 disruption, whereas they are resistant to conventional heating.

659 The distribution of compounds released from microwave irradiation in KOH indicates an  
660 important suberin- and cutin-derived contribution to the ROM. Moreover, this distribution,  
661 together with the value of the suberin/cutin ratio suggests a significant input of both cutin and  
662 suberin from the undergrowth.

663 Lignin-derived compounds, present in all the extracts from microwave hydrolyses are  
664 dominated by guaiacyl moieties, in agreement with the predominance of pines at the study  
665 site. A significant contribution from the non-woody angiosperm undergrowth to the ROM is  
666 also suggested by the presence of *p*-hydroxyphenyl units (mainly *p*-coumaric acid) together  
667 with that of cyclodimers of *p*-coumaric acid. Two types of lignin monomers are shown to be  
668 engaged in different linkages (ether vs ester) as they are specifically released through  
669 microwave HCl or KOH hydrolyses.

670 The composition of the CuPy and CuTHM pyrolysates of the residues obtained after  
671 microwave irradiations were consistent with the distribution patterns of the corresponding  
672 extracts.

673 Finally, the present study illustrates how complementary the microwave irradiation and  
674 pyrolysis methods are. Each method presents its own advantages and drawbacks. Microwave  
675 assisted extractions and hydrolyses afford quantitative (e.g. yield of degradation) as well as  
676 qualitative data (e.g. nature of constituent monosaccharides) which cannot be obtained  
677 through on-line pyrolysis. In addition, this technique appears to provide a more accurate  
678 measure of the contribution of cutin-derived constituent (i.e. mid-chain hydroxy alkanolic  
679 acids) due to the incomplete methylation of the hydroxyl groups occurring during CuTHM  
680 pyrolysis. On the other hand, contrary to pyrolysis, microwave assisted hydrolyses appears to  
681 be unable to release the major part of the lignin-derived constituent of the ROM. On-line  
682 pyrolysis is much less time consuming. However, it is necessary to perform both CuPy and  
683 CuTHM to have a holistic view of the composition of the ROM (e.g. carbohydrate only  
684 detected in CuPy pyrolysate and polar constituents only detected in CuTHM pyrolysate).  
685 Microwave assisted extractions and/or hydrolyses appear as particularly suitable for the  
686 analysis of complex matrices and could be an attractive and complementary technique to  
687 pyrolysis methods.

688

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690

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698

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- 836

837 Table 1

838

839 Compounds identified in the extracts from the sequential microwave irradiation treatments

840 (black numbers in Figure 2).

841

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<b>1</b>	4-hydroxybenzaldehyde
<b>2</b>	3-hydroxybenzoic acid
<b>3</b>	3(4)-hydroxyacetophenone
<b>4</b>	4-hydroxybenzoic acid
<b>5</b>	4-hydroxy-3-methoxybenzaldehyde
<b>6</b>	4-hydroxy-3-methoxyacetophenone
<b>7</b>	4-hydroxy-3-methoxybenzoic acid (vanillic acid)
<b>8</b>	3-(4-hydroxy-3-methoxyphenyl)-propan-2,3-dione (see Fig. 3a)
<b>9</b>	3,4-dihydroxybenzoic acid
<b>10</b>	3,5-dihydroxybenzoic acid
<b>11</b>	4-hydroxy-2,6-dimethoxyacetophenone
<b>12</b>	3-(4-hydroxyphenyl)-2-propenoic acid ( <i>p</i> -coumaric acid)
<b>13</b>	4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)
<b>14</b>	3-(4-hydroxy-3-methoxyphenyl)-3-oxopropanol (see Fig. 3b)
<b>15</b>	3-(4-hydroxy-3-methoxyphenyl)-3-oxo-1-propen-1(2)-ol (see Fig. 3c)
<b>16</b>	3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid)
<b>17</b>	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3d)
<b>18</b>	unidentified (see Fig. 3e)
<b>19</b>	2-(4-hydroxy-3,5-dimethoxyphenyl)-2-oxo-ethanoic acid (see Fig. 3f)
<b>20</b>	Cyclodimers of <i>p</i> -coumaric acid (see Fig. 3g)
<b>21</b>	9-hydroxy pentadecanoic acid
<b>22</b>	10-hydroxy pentadecanoic acid
<b>23</b>	8/9/10-hydroxy octadecanoic acid
<b>24</b>	6/7 hydroxy pentadecanedioic acid
<b>25</b>	9,15-dihydroxy pentadecanoic acid
<b>26</b>	7/8-hydroxy hexadecanedioic acid
<b>27</b>	8/9/10,16-dihydroxy hexadecanoic acid
<b>28</b>	9,10-dihydroxy octadecanoic acid
<b>29</b>	9/10/11,18-dihydroxy octadecanoic acid
<b>30</b>	9,10,18-trihydroxy octadecanoic acid

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843

844

845  
 846 Table 2  
 847 Compounds identified in the 610 °C Curie point pyrolysates of the ROM and residues  
 848 remaining after microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers  
 849 in Figure 4)

850  
 851

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<b>1</b>	2-furancarboxaldehyde
<b>2a + 2b</b>	benzaldehyde + methylfurancarboxaldehyde <sup>a</sup>
<b>3</b>	phenol
<b>4</b>	acetophenone
<b>5</b>	2-methoxyphenol
<b>6</b>	levoglucosenone
<b>7</b>	4-methyl-2-methoxyphenol
<b>8</b>	dihydrobenzofuran
<b>9</b>	4-ethenyl-2-methoxyphenol
<b>10</b>	3-methoxy-4-hydroxybenzaldehyde
<b>11</b>	4-(propen-2-yl)-2-methoxyphenol
<b>12</b>	3-methoxy-4-hydroxyacetophenone
<b>13</b>	3-methoxy-4-hydroxybenzoic acid methyl ester
<b>14</b>	3-methoxy-4-hydroxyphenylacetone
<b>15</b>	levoglucosan
<b>16</b>	3,4-dimethoxyphenylacetone <sup>b</sup>

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852  
 853  
 854 <sup>a</sup> The ratio of **2b/2a** estimated through the intensity of peaks at  $m/z$  110 and 106 decreases  
 855 from ca. 1.8 for the ROM to ca. 0.9 and 0.2 for the residues from microwave hydrolyses in 0.1  
 856 M and 1 M HCl respectively. <sup>b</sup> tentatively identified ( $m/z$  123, 151 (base peak), 194)  
 857

858

859 Table 3

860

861 Compounds identified in the 610 °C Curie point thermally assisted hydrolysis and

862 methylation pyrolysates of the ROM and residues remaining after microwave irradiation in

863 0.1 M HCl, 1 M HCl and 1 M KOH (black numbers in Figure 5)

864

865

866

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<b>1</b>	methoxy toluene		
<b>2</b>	2-methoxyphenol		
<b>3</b>	benzoic acid		
<b>4</b>	1,2-dimethoxybenzene		
<b>5</b>	4-methoxy ethenylbenzene		
<b>6</b>	3,4-dimethoxytoluene		
<b>7</b>	4-methoxybenzaldehyde		
<b>8</b>	1,2,3-trimethoxybenzene		
<b>9</b>	3-methoxybenzoic acid		
<b>10</b>	{ 3,4-dimethoxy ethenylbenzene 1,2,4-trimethoxybenzene 4-methoxybenzoic acid		
		<b>11</b>	3,4,5-trimethoxy ethenylbenzene
		<b>12</b>	3,4-dimethoxybenzaldehyde (vanillic aldehyde)
<b>13</b>	3,4-dimethoxyacetophenone (acetovanillone)		
<b>14</b>	3,4-dimethoxybenzoic acid (vanillic acid)		
<b>15</b>	3,4,5-trimethoxybenzaldehyde (syringaldehyde)		
<b>16</b>	3,4-dimethoxybenzeneacetic acid		
<b>17</b>	3-(4-methoxyphenyl)-2-propenoic acid ( <i>p</i> -coumaric acid)		
<b>18</b>	3,4,5-trimethoxybenzoic acid (syringic acid)		
<b>19</b>	3-(3,4-dimethoxyphenyl)-2-propenoic acid (ferulic acid)		
<b>20</b>	9/10,16-dimethoxy hexadecenoic acid (Fig. 6a)		
<b>21</b>	8/9/10,16-dimethoxy hexadecanoic acid (Fig. 6b)		
<b>22</b>	7/8/9-methoxy-15-hydroxy pentadecanoic acid (Fig. 6c)		
<b>23</b>	7/8-dimethoxy hexadecanedioic acid (Fig. 6d)		
<b>24</b>	8/9-methoxy-16-hydroxy hexadecanoic acid (Fig. 6e)		
<b>25</b>	9,18-dimethoxy octadecanoic acid (Fig. 6f)		
<b>26</b>	9/10-hydroxy-18-methoxy octadecanoic acid (Fig. 6g)		
<b>27</b>	cyclodimers of <i>p</i> -coumaric acid		

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867

868

869

870 Table 4

871

872  $\omega$ -Hydroxy acid/*n*-alkanoic acid,  $\omega$ -hydroxy acid/*n*-alkan-1-ol and *n*-alkan-1-ol I/*n*-alkanoic  
873 acid ratios calculated from the peak areas of GC-MS traces of CuTHM pyrolysates.

874

875

876

	ROM	Residue after microwave irradiation in 1 M HCl	Residue after microwave irradiation in 1 M KOH
C <sub>22</sub> $\omega$ -hydroxy acid/C <sub>22</sub> <i>n</i> -alkanoic acid	7.4	2.4	2.4
C <sub>22</sub> $\omega$ -hydroxy acid/C <sub>22</sub> <i>n</i> -alkan-1-ol	2.3	1.7	2.1
C <sub>22</sub> <i>n</i> -alkan-1-ol I/ C <sub>22</sub> <i>n</i> -alkanoic acid	3.2	1.4	1.1
C <sub>24</sub> $\omega$ -hydroxy acid/C <sub>24</sub> <i>n</i> -alkanoic acid	4.6	1.5	1.6
C <sub>24</sub> $\omega$ -hydroxy acid/C <sub>24</sub> <i>n</i> -alkan-1-ol	2.6	1.7	1.9
C <sub>24</sub> <i>n</i> -alkan-1-ol I/ C <sub>24</sub> <i>n</i> -alkanoic acid	1.8	0.9	0.8

877

878

879

880 Figure Captions

881

882 Fig. 1. Sequential microwave irradiation treatments of the ROM from the forest soil sample.

883

884 Fig. 2. Total ion current (TIC) traces of the extracts from microwave irradiation in (a) H<sub>2</sub>O;  
885 (b) 0.1 M HCl; (c) 1 M KOH. The compounds were identified as methyl ester and  
886 trimethylsilyl ether derivatives. Black numbers correspond to compounds listed in Table 1.  
887 White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-1-ols; ●:  
888 ω-hydroxy acids; ▽: *n*-alkanedioic acids; **S**: mono- or disaccharides; \* : pollutant. When two  
889 different peaks are labelled with the same number (e.g. **7** or **9**), that with the highest retention  
890 time corresponds to the trimethylsilyl ester and ether derivative.

891

892 Fig. 3. Mass spectra of tentatively identified and unidentified aromatic compounds found in  
893 the organic extracts from the microwave extractions and hydrolyses (Table 1 for peak  
894 annotations). The analysis of compounds as trimethylsilyl ester and ether derivatives reveals  
895 (i) two compounds with the same retention times and mass spectra as compounds **8** and **15**,  
896 indicating the absence of carboxyl groups in these compounds. (ii) a compound, at longer  
897 retention time than compound **14**, with a mass spectrum exhibiting peaks at *m/z* 193, 223  
898 (base peak), 325, 340 and 383. This suggests the presence of one carboxyl group for  
899 compound **14**. Compound **17** has the molecular weight of a trimethylsilyl ether, trimethylsilyl  
900 ester of methoxyhydroxybenzoic acid or trimethylsilyl ether, methyl ester of  
901 dihydroxybenzoic acid. However the mass spectra of these latter compounds do not match  
902 with that of compound **17**. Compounds **18** and **19** have the molecular weight of a  
903 trimethylsilyl ether, trimethylsilyl ester of dihydroxybenzoic acid, but no match was found  
904 between the mass spectra of these different compounds.

905

906

907 Fig. 4. TIC traces of 610 °C Curie point (CuPy) pyrolysate of (a) ROM and residues  
908 remaining after microwave irradiation in (b) 0.1 M HCl; (c) 1 M HCl; (d) 1 M KOH. Black  
909 numbers correspond to compounds listed in Table 2. White numbers correspond to carbon  
910 chain length. ▼: *n*-alkanoic acids; ◇: *n*-alkane/*n*-alkene doublets; ◆: *n*-alkan-2-one/*n*-alken-  
911 2-one doublets.

912

913 Fig. 5. TIC traces of 610 °C Curie point thermally assisted hydrolysis and methylation  
914 (CuTHM) pyrolysate of (a) ROM and residues remaining after microwave irradiation in (b)  
915 0.1 M HCl; (c) 1 M HCl; (d) 1 M KOH. Black numbers correspond to compounds listed in  
916 Table 3. White numbers correspond to carbon chain length. ▼: *n*-alkanoic acids; ○: *n*-alkan-  
917 1-ols; ●: ω-hydroxy acids; ▽: *n*-alkanedioic acids; ◇: *n*-alkane/*n*-alkene doublets; \*:  
918 hexahydro-1,3,5-trimethyl-1,3,5-triazine, a by product from TMAH.

919

920 Fig. 6. Mass spectra of the tentatively identified mid-chain substituted ω-hydroxy alkanolic  
921 and alkanedioic acids found in the CuTHM pyrolysates of the ROM and residues from  
922 microwave irradiation in 0.1 M HCl, 1 M HCl and 1 M KOH. (Table 3 for peak annotation).

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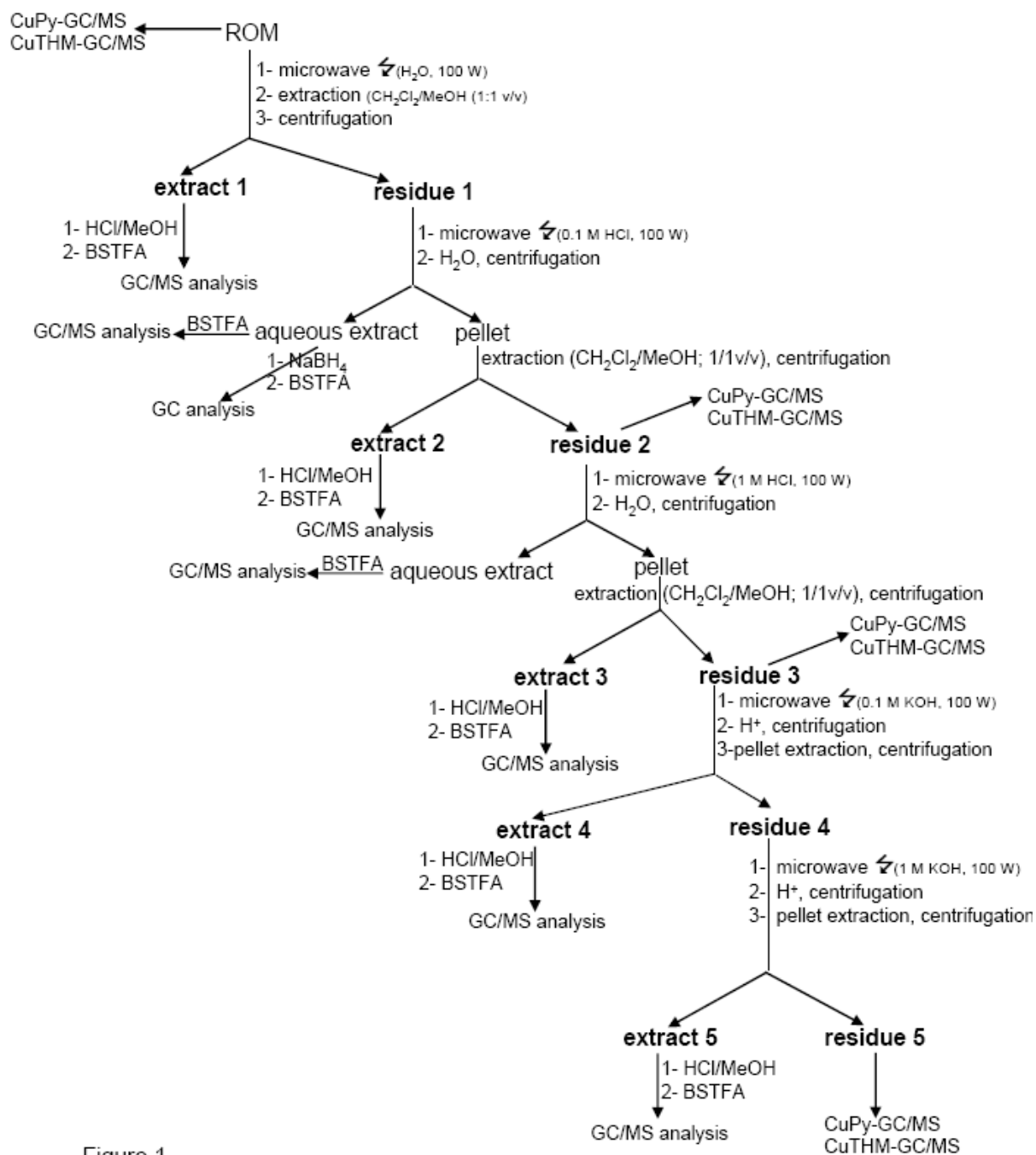


Figure 1

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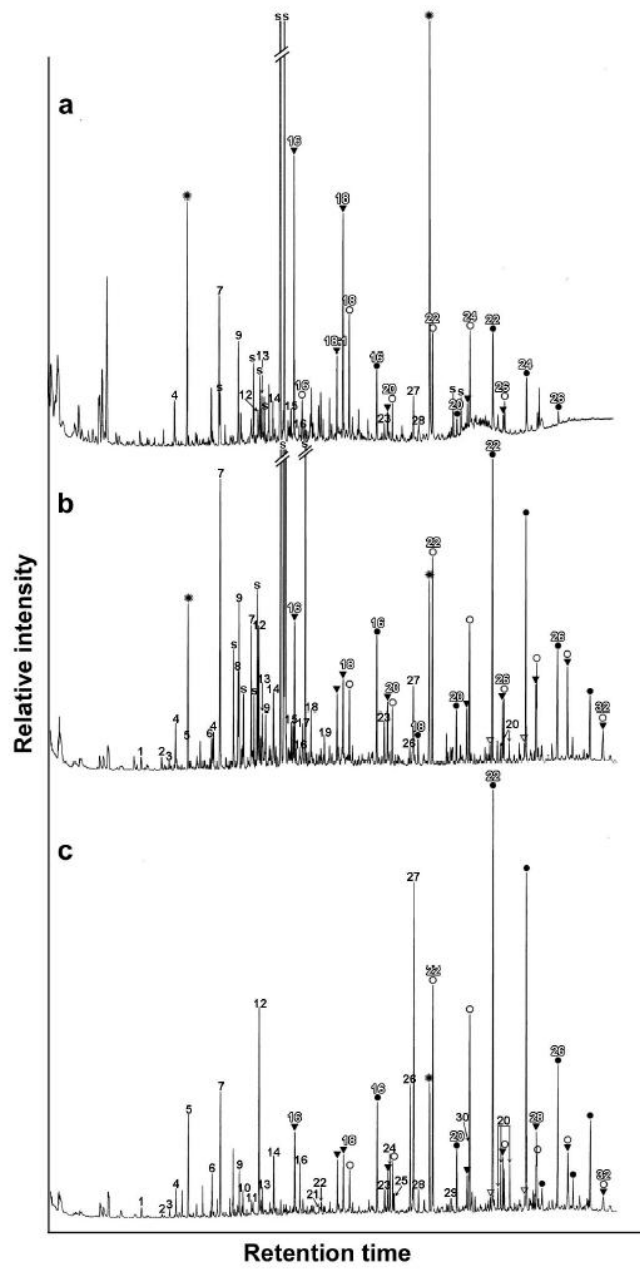


Figure 2

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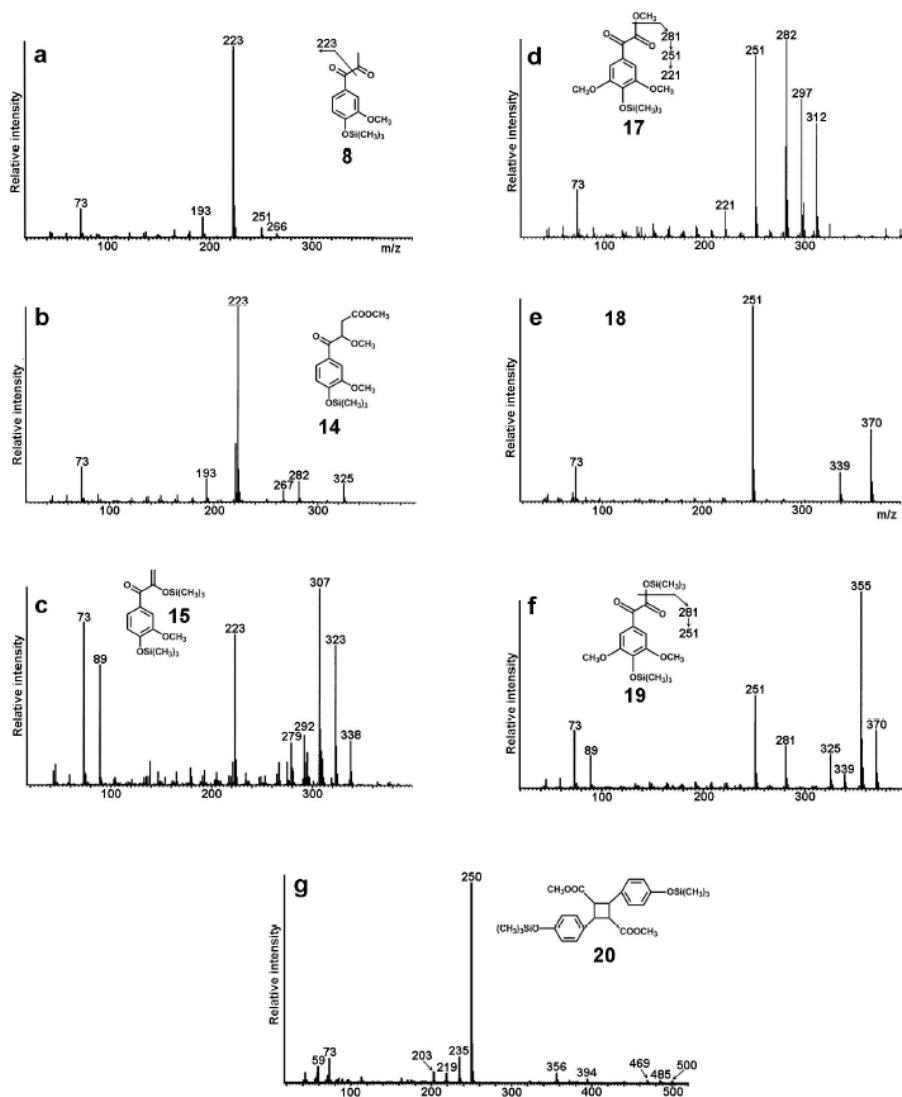


Figure 3

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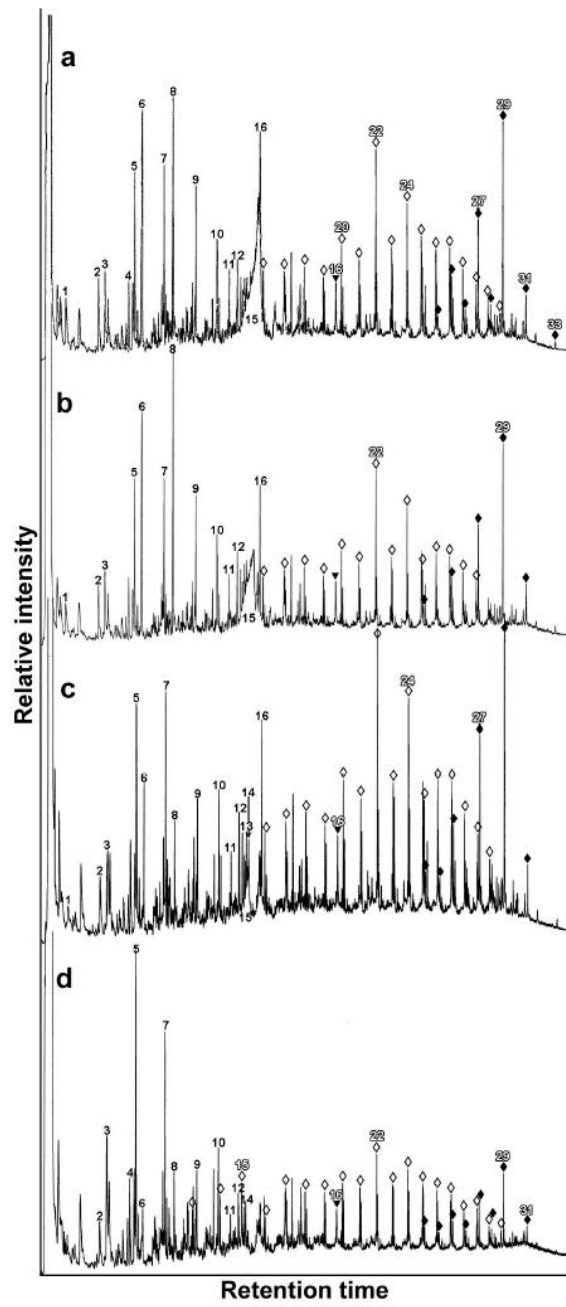


Figure 4

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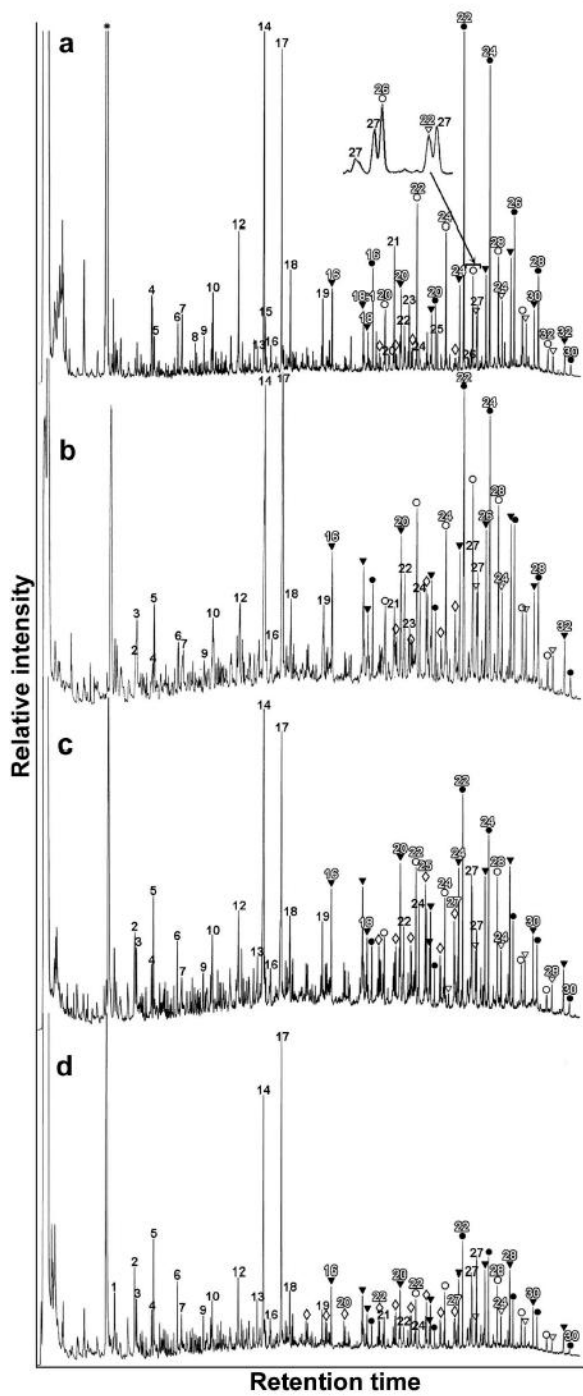


Figure 5

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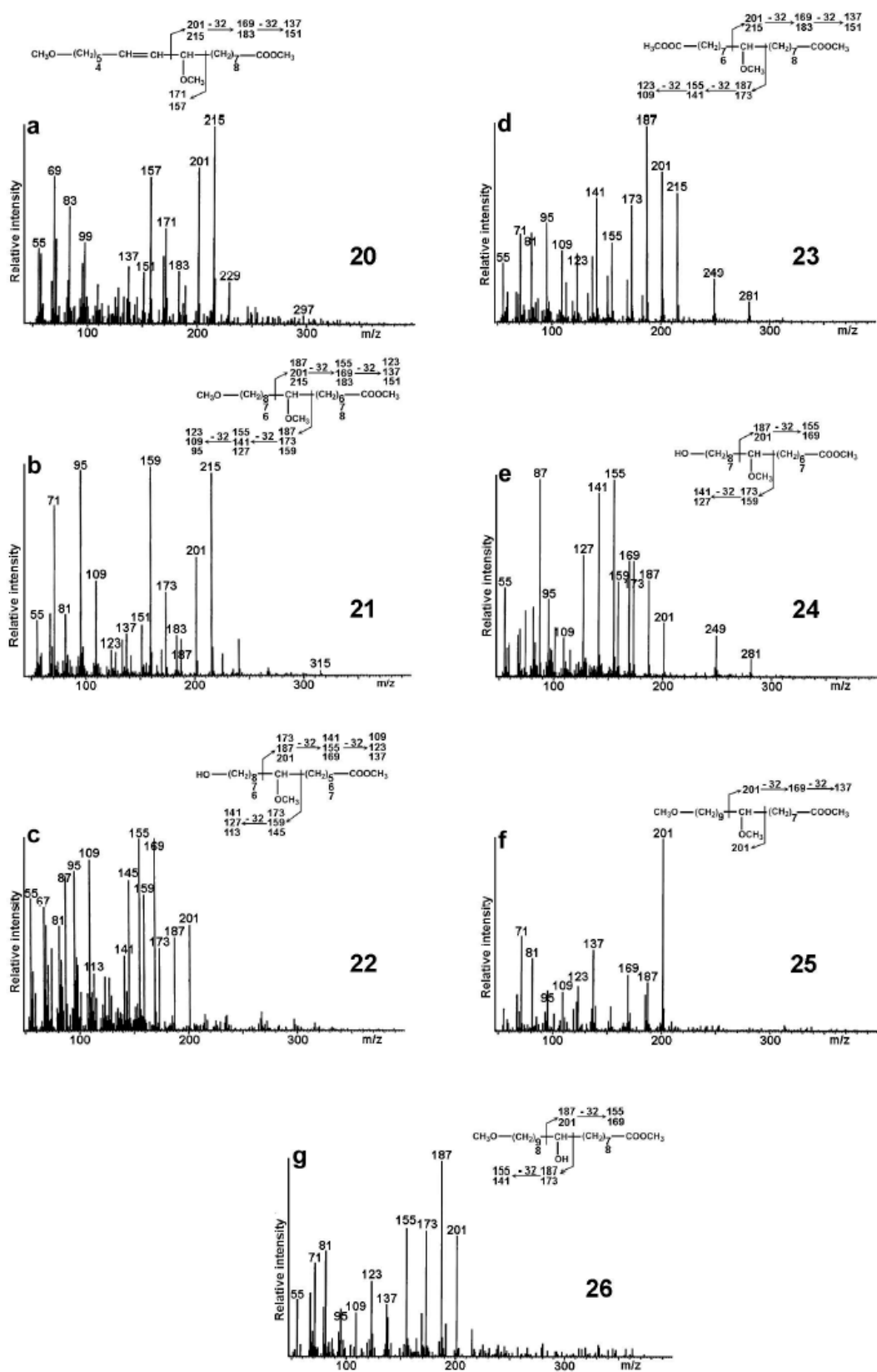


Figure 6